milliliter. Transfer 10 milliliters of the standard copper solution to a 60-milliliter separatory funnel.

(iii) *Preparation of the sample.* Accurately weigh approximately 15 milligrams of sample into a 60-milliliter separatory funnel. Dissolve the sample in 10 milliliters of 0.1*N* hydrochloric acid.

(iv) *Procedure.* To the separatory funnels containing the sample solution and standard copper solution, add 10 milliliters of the zinc dibenzyldithiocarbamate solution and shake the funnels vigorously for 1 minute. Allow the phases to separate. Filter the carbon tetrachloride phase (lower phase) through 1 gram of anhydrous sodium sulfate to remove excess water. Using a suitable spectrophotometer equipped with 1-centimeter cells, and carbon tetrachloride as a blank, measure the absorbance of the standard copper solution and the sample solution at 435 nanometers. Calculate the percent copper as follows:

$$\text{Percent copper} = \frac{\text{Absorbance of sample solution} \times 1.5}{\text{Absorbance of standard copper solution} \times \text{Sample weight in milligrams}}$$

(9) *Content of various bleomycin fractions.* Proceed as directed in § 436.339 of this chapter.

(10) *Identity test.* Proceed as directed in § 436.211 of this chapter, using the method described in paragraph (b)(1) of that section, using a 1 percent mixture.

[40 FR 52005, Nov. 7, 1975; 40 FR 53998, Nov. 20, 1975, as amended at 46 FR 60568, Dec. 11, 1981; 48 FR 51913, Nov. 15, 1983; 50 FR 19920, May 13, 1985]

§ 450.20 Dactinomycin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Dactinomycin is a bright-red compound that is so purified and dried that:

(i) Its dactinomycin content is not less than 900 micrograms of dactinomycin per milligram, calculated on an anhydrous basis.

(ii) Its loss on drying is not more than 15 percent.

(iii) Its absorptivity at 445 nanometers is not less than 0.95 and not more than 1.03 times that of the dactinomycin working standard at the same wavelength. Its absorbance at 240 nanometers is not less than 1.3 and not more than 1.5 times its absorbance at 445 nanometers.

(iv) It is crystalline.

(v) It passes the identity test for dactinomycin.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5(b) of this chapter, and in addition each package shall bear on its label the statement ''Protect from light and excessive heat.''

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for dactinomycin content, loss on drying, absorptivity, crystallinity, and identity.

(ii) Samples required: 16 packages, each containing approximately 40 milligrams.

(b) *Tests and methods of assay.* Dactinomycin is toxic and corrosive. It must be handled with care in the laboratory. Transfer all dry powders in a suitable hood, while wearing rubber gloves. Avoid inhaling fine particles of the powder. Do not pipette by mouth. If any of the substance contacts the skin, wash copiously with soap and water. Dispose of all waste material by dilution with large volumes of trisodium phosphate solution.

(1) *Dactinomycin content.* Proceed as directed in § 436.331 of this chapter, preparing the sample and calculating the dactinomycin content as follows:

(i) *Preparation of sample solution.* Accurately weigh a sufficient amount of the sample to obtain a solution containing approximately 0.25 milligram per milliliter of dactinomycin in mobile phase.

(ii) *Calculations.* Calculate the micrograms of dactinomycin per milligram of sample as follows:

$$\text{Micrograms of dactinomycin per milligram} = \frac{A_u \times P_s \times 100}{A_s \times C_u \times (100 - m)}$$

where:

A_u = Area of the dactinomycin peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s = Area of the dactinomycin peak in the chromatogram of the dactinomycin working standard;

P_s = Dactinomycin activity in the dactinomycin working standard solution in micrograms per milliliter;

C_u = Milligrams of sample per milliliter of sample solution; and

m = Percent moisture content of the sample.

(2) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(3) *Absorptivity*—(i) *Procedure.* Accurately weigh approximately 15 milligrams of the sample ''as is'' and 15 milligrams of the working standard dried as directed in § 436.200(a) of this chapter. Transfer each weighing to separate 100-milliliter volumetric flasks. Dissolve the material and bring to volume with spectrophotometric-grade methyl alcohol. Mix well. Pipette 5.0 milliliters of each solution into separate 25-milliliter volumetric flasks, dilute to volume with spectrophotometric-grade methyl alcohol. Mix well. Using a suitable spectrophotometer and 1-centimeter absorption cells, determine the absorbance of the sample solution at the 240-nanometer and at the 445-nanometer absorption peaks (the exact position of the peaks should be determined for the particular instrument used). Determine the absorbance of the standard at the 445-nanometer absorption peak.

(ii) *Calculations.* Calculate the relative absorptivity and the ratio for the absorbances of the sample as follows:

$$\text{Relative absorptivity at 445 nanometers} = \frac{A_2 \times \text{milligrams of standard} \times \text{potency of the standard in micrograms per milligram}}{A_3 \times \text{milligrams of sample} \times (100 - M) \times 10}$$

$$\text{Ratio for the absorbances of the sample at 240 and 445 nanometers} = \frac{A_1}{A_2}$$

where:

A_1=Absorbance at 240 nanometers for the sample;

A_2=Absorbance at 445 nanometers for the sample;

A_3=Absorbance at 445 nanometers for the standard;

M=Percent moisture in the sample.

(4) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

(5) *Identity.* The high-pressure liquid chromatogram of the sample determined as directed in paragraph (b)(1) of this section compares qualitatively to that of the dactinomycin working standard.

[49 FR 6092, Feb. 17, 1984, as amended at 49 FR 24018, June 11, 1984; 50 FR 19675, May 10, 1985]

§ 450.22 Daunorubicin hydrochloride.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Daunorubicin hydrochloride is the monohydrochloride salt of (1*s*,3*s*)-3-acetyl-1,2,3,4,6,11-hexahydro-3,5,12-trihydroxy-10-methoxy-6,11-dioxo-1-naphthacenyl-3-amino-2,3,6-trideoxy-α-L-*lyxo*-hexopyranoside. It is a red-orange, hygroscopic powder. It is so purified and dried that:

(i) Its potency is not less than 842 micrograms and not more than 1,030 micrograms of daunorubicin per milligram.

(ii) Its moisture content is not more than 3.0 percent.

(iii) Its pH in an aqueous solution containing 5 milligrams per milliliter is not less than 4.5 and not more than 6.5.

(iv) It is crystalline.

(v) It passes the identity test for daunorubicin.